CHAPTER 2

LITERATURE REVIEW

1. Skin morphology

The skin is composed of three primary layers: epidermis, dermis, and subcutaneous tissue. Each layer possesses specific characteristics and functions. The epidermis is the outermost layer of the skin. It is very important from a cosmetic stand point, because it is this layer that gives the skin its texture and moisture, and contributes to skin color. If the surface of the epidermis is dry or rough, the skin appears aged. In order of abundance, the composition of skin surface lipids includes triglycerides, fatty acids, squalene, wax esters, diglycerides, cholesterol esters, and cholesterol (Baumann, 2009). These lipids are an integral part of the epidermis.

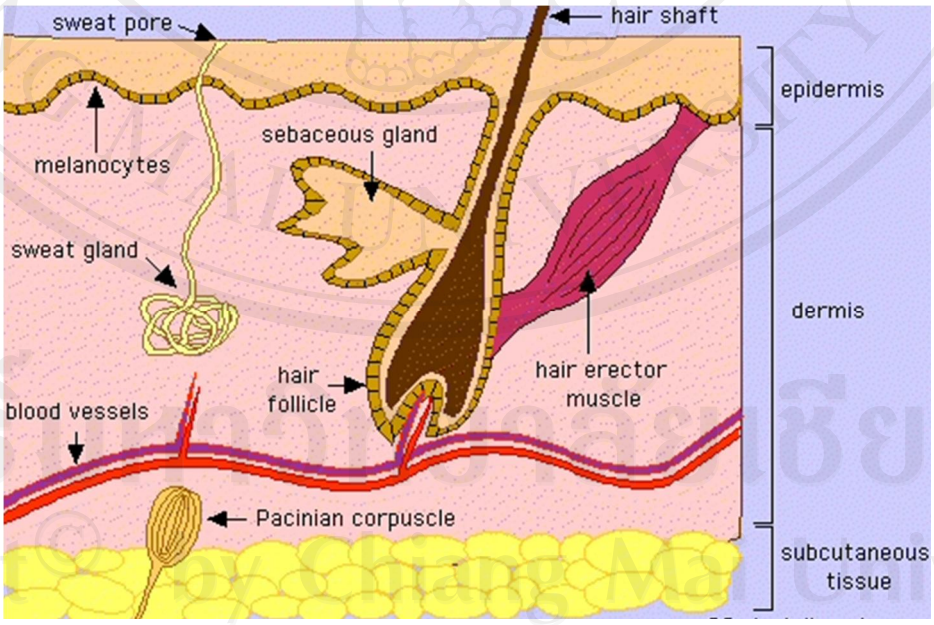

Figure 2-1 The three layers of the skin (http://www.enchantedlearning.com/subjects/anatomy/skin/, available online 12/12/2012).

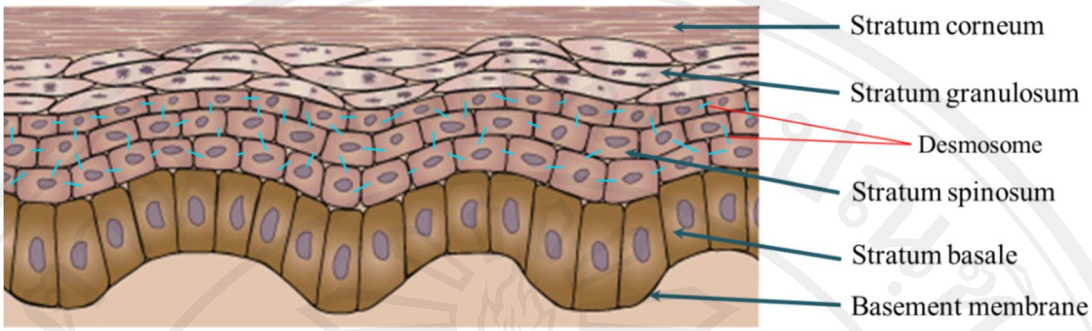

Figure 2-2 The layers of the epidermis (modified after http://dermatology.about.com/od/anatomy/ss/epidermis.htm, available online 12/12/2012).

The most superficial layer of the epidermis is the stratum corneum (SC), also known as the horny layer. SC is a condensed mass of the cells that have lost their nuclei and granules and deposits on average approximately 15 cell layers thick. It functions as a protective barrier, which the main function is to prevent transepidermal water loss and regulate the water balance in the skin. The two major components that allow the SC to perform this role are lipids and the natural moisturizing factor. The major lipids found in the SC that contribute to the water permeability barrier are ceramides, cholesterol, and fatty acids (Puglia and others, 2008). Deficiency of these lipids predisposes the individual to dry skin. SC lipids can be affected by age, genetics, seasonal variation, and diet.

2. Traditional carrier systems

Many actives used in the pharmaceutical and cosmetic industries are chemically labile and difficult to stabilize in the final product. It is known that the incorporation of chemically labile substances in colloidal carriers, such as liposomes, emulsions, and polymeric nanoparticles, can improve drug stability (Jee and others, 2006; Kristl and others, 2003).

2.1. Liposome

The liposomes were re-invented by Bangham in 1968. It took almost 20 years before the first cosmetic product appeared on the market in 1986. The name was "capture" and it was launched by the company Dior. With liposomes, the lipophilic actives are localized in the phospholipids bilayer. These actives can also partition to the water phase. Hydrophilic actives dissolved in the water core of the liposomes are in exchange with the external water phase. Moreover, liposomes are susceptible to hydrolysis and oxidation due to the contents of unsaturated fatty acids and ester bonds in their phospholipid bilayers (Grit and others, 1993). This limits the long-term stability of liposomes. Therefore, liposome is the lack of protection for chemically labile drugs, in addition, drug release takes place at least relatively fast. The drawback also came from the high cost of liposomes.

2.2. Emulsion

An emulsion is a thermodynamically unstable system consisting of at least two immiscible liquid phase, one of which is dispersed as globules (the dispersed phase) in the outer liquid phase (the continuous phase), stabilized by the presence of an emulsifying agent (Rousseau, 2000; Sinko, 2006). The system, which the oil phase is dispersed as globules throughout an aqueous continuous phase, is called oil-in-water (o/w) emulsion. On the other hand, the system is referred to as a water-in-oil (w/o), when the oil phase serves as the continuous phase. Several methods can be used to prepare the emulsion such as phase inversion temperature method, low-energy emulsification methods, high-energy emulsification methods (Anton and others, 2008). Pharmaceutical applications of emulsion can be applied for oral, dermal and intravenous administrations. Macroemulsion requires some energy to form. It is milky

and nonstable, whereas NE is transparent, stable, and spontaneously formed (Pathak and Thassu, 2009). A co-surfactant is commonly used to lower the interfacial tension and fluidize the interfacial surfactant (Heuschkel and others, 2008; Kogan and Garti, 2006). The drawback of NE is a high proportion of surfactants and the physical stability problems. Due to the liquid state of oil droplets in o/w emulsions, relatively fast partitioning of lipophilic actives between oil phase and water takes place (Yoon and Burgess, 1998).

2.3. Polymeric nanoparticles

The polymeric nanoparticles were invented in the middle of the seventies, originally intended for pharmaceutical use. They are possible carriers for controlled drug delivery and targeting by the intravenous route (Davis, 1981) and possess the advantage of a solid matrix allowing flexibility in the modification of drug release and protecting incorporated drugs against chemical degradation. After many years there was limited use in cosmetic industry, but even after 30 years of intensive research and many thousands of scientific publications no pharmaceutical product for the therapy is on the market. The problems are manifold. They are temperature sensitive. That means they tend to stick at elevated temperatures and cannot be heat sterilized (autoclaved). There is lack of regulatory accepted polymers; newly synthesized polymers have many nice features, but require expensive toxicity studies before acceptance by the regulatory authorities. Other problems are also the cost of production, especially the lack of large-scale production methods able to be qualified, validated, and leading to a product being acceptable to the regulatory authorities. There are interesting data in academic research. However, there is a lack of concepts

to transfer these successes to the industrial scale, and final products with some benefits to the patient.

3. Innovative carrier systems

3.1. Lipid nanoparticles

Solid lipid nanoparticle (SLN) is a particle made from solid lipids, and stabilized by emulsifying agents (Mehnert and Mader, 2001). It is an alternative carrier system to traditional systems in the first generation which is developed at the beginning of the 1990s. Many carrier systems are provided such as virosome, neosome, liposome, microemulsion (Ghosh and Murthy, 2006; Schäfer-Korting and others, 2007). SLN is combined advantages of the traditional systems, but avoided the major disadvantages of them. All excipients are accepted as GRAS status, well tolerance, and biocompatible, thus SLN is safe. It can be produced by HPH with hot or cold homogenization techniques. SLN is available for large scale production and well known in pharmaceutical industry (Müller and others, 2000a). There are many pharmaceutical applications for lipid nanoparticles such as dermal, peroral, ocular, parenteral, pulmonary administration. SLN alone can act as a physical sunscreen by reflect and scatter UV-radiation. Wissing and Müller (2002) incorporated oxybenzone in SLN, they also found that SLN incorporated with molecular sunscreen leads to synergistic UV-blocking behavior. Besides, SLN can also increase skin hydration due to lipid film formation onto the skin. It was confirmed by the *in vivo* studied by Pardeike *et al*. (2009) which found that cream containing SLN significantly increases skin hydration. The occlusion factors of lipid nanoparticles depend on the particle size and concentration. The occlusion can be increased by decreasing the particle size (at a given lipid concentration) or alternatively at a given particle size by increasing the

number of particles (concentration of lipid). Therefore, for lipid nanoparticles a "controlled occlusion effect" can be claimed (Figure 2-3). Souto *et al*. (2005b) made a novel approach on α-lipoic acid delivered to skin *via* solid lipid nanoparticle by incorporated α-lipoic acid in SLN. Alpha-lipoic acid is an anti-aging compound which is chemically labile and easily to degrade, thus, it possesses an unpleasant odor. This active compound is protected against chemical degradation by SLN.

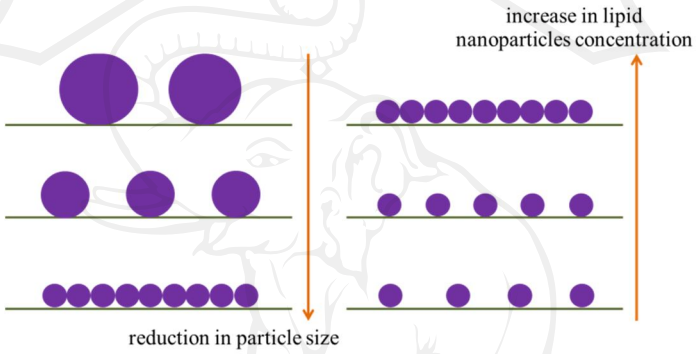

Figure 2-3 The controlled occlusion effect of lipid nanoparticles is a function of the particle size (left: at identical lipid concentration, one big particle gives many small particles) and a function of increasing particle number (right: increase of lipid concentration, at a given particle size).

In the second generation (Figure 2-4), lipid nanoparticles are blends of solid lipid and liquid lipid or so-called the nanostructured lipid carriers (NLC) (Müller, 2007; Müller and Dingler, 2007, Müller and others, 2007). It has the benefits overcome SLN in the improving of release properties (Müller and others, 2002b). Moreover, drug expulsion during storage are minimized or avoided by NLC. Souto and Müller (2006) successfully incorporated clotrimazole into SLN and NLC, thereby, they increased its dermal bioavailability and potentially reduced its side

effects. Pardeike *et al*. (2009) also summarized on the review article focus on lipid nanoparticles for dermal application that NLC showed a higher loading capacity of active compound. As developed from SLN, NLC can be produced by various techniques, however, the preferred production method is HPH the same as SLN. This is due to the large scale production of NLC is easily possible (Radtke and others, 2005). Müller *et al.* (2002a) reported that there were three types of NLC which were imperfect type, structureless type, and multiple type. Teeranachaideekul *et al.* (2007a; 2007b) studied on NLC composed of cetylpalmitate with various amounts of triacylglycerol incorporated with coenzyme Q10. They found that the increase of oil loading did not affect the particles size of NLC formulations. However, increasing the amounts of oil could lead to a less ordered structure within the particles. NLC showed a fast release initially for skin saturation followed by a slow and prolonged release to maintain the concentration of coenzyme Q10 in skin. They concluded that NLC has been proven to be a suitable carrier for active pharmaceuticals especially for the topical route. Both SLN and NLC are attractive alternative carrier systems for topical cosmeceutical products. However, NLC is a smarter system at this moment.

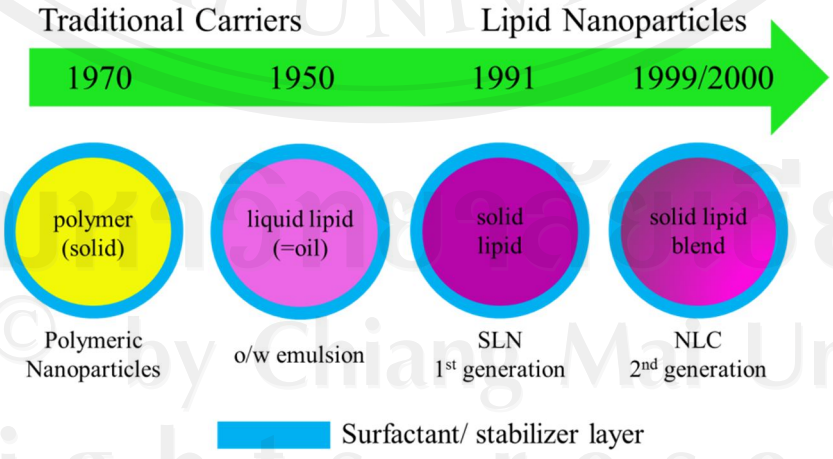

Figure 2-4 Development of traditional carriers to lipid nanoparticles.

Due to their small size, upon dermal application lipid nanoparticles adhere to the lipid film of the SC. Lipids adhere well to lipids (hydrophobic interactions). The adsorption film repairs bare patches in the lipid film of the skin, and thus reinforces a very thin film. This restoration of the protective lipid film increases the skin hydration, and normalizes the living conditions for the cells underneath. An increased skin hydration also reduces wrinkles as positively side effect, which is especially desired in cosmetic products. The film has also some occlusive properties, which promotes penetration of many drugs into the skin (Müller and other, 2011). Figure 2-5 and Figure 2-6 summarizes the properties of lipid nanoparticles on the skin. In areas where the lipid film of the SC is damaged or disappeared, the particles can restore the lipid film.

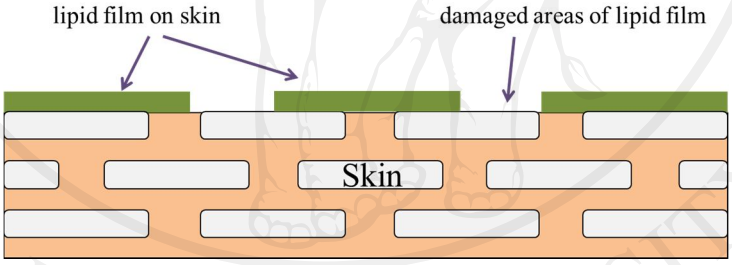

Figure 2-5 Situations on damaged skin.

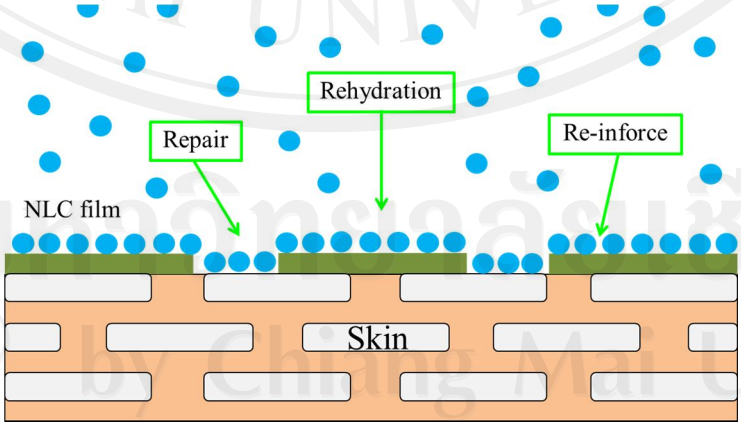

Figure 2-6 Propose mechanisms of lipid nanoparticles (SLN and NLC) in repairing the damaged skin.

The first product based on lipid nanoparticles (Nanobase®, Yamanouchi) was introduced to the market in Poland and is patent protected (de Vringer, 1992). Nanobase® exploits the special properties of placebo SLN, such as good application properties and adhesion leading to skin hydration. More recently, NLC have also reached the market. The NLC were developed in 1999/2000, the first two products were launched only 5 years later by Dr. Rimpler GmbH. The product Nanolipid CLR Restore is the third product within about half a year. Examples of cosmetic products currently on the market containing NLC were summarized in Table 2-1.

Table 2-1 Overview of NLC-based cosmetic products introduced to the market since October 2005 onward.

Product name	**Producer**	**Market introduction**	**Main active ingredients**
Cutanova Cream Nano Repair Q10	Dr. Rimpler	10/2005	Q 10, polypeptide, hibiscus extract, ginger extract, ketosugar
Intensive Serum Nano Repair Q10		10/2005	Q 10, polypeptide, mafane extract
Cutanova Cream NanoVital Q10		06/2006	Q 10, TiO_2, polypeptide, ursolic acid, oleanolic acid, sunflower seed extract
Dr. Cutanova Cream Nanosensitive forte		10/2008	
Dr. Cutanova Emuslion Nanosensitive		10/2008	
SURMER	Isabelle Lancray		
- Crème Legère Nano-Protection		11/2006	kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, milk extract from coconut, wild indigo, noni extract
- Crème Riche Nano-Restructurante		11/2006	
- Elixir du Beauté Nano-Vitalisant		11/2006	
- Masque Crème Nano-Hydratant		11/2006	
- Crème Contour Des Yeux Nano Remodelante		03/2008	kukuinut oil, Monoi Tiare Tahiti®, pseudopeptide, hydrolized wheet protein
NanoLipid	Chemisches Laboratorium Dr. Kurt Richter, (CLR)		
- Restore CLR		04/2006	black currant seed oil containing ω-3 and ω-6 unsaturated fatty acids
- Q10 CLR		07/2006	Q10 and black currant seed oil
- Basic CLR		07/2006	caprylic/capric triglycerides
- Repair CLR		02/2007	black currant seed oil and manuka oil

Table 2-1 (Cont.)

Product name	Producer	Market introduction	Main active ingredients
IOPE SuperVital	Amore Pacific	09/2006	Q10, ω-3 und ω-6 unsaturated fatty acids
- Cream			
- Serum			
- Eye cream			
- Extra moist softener			
- Extra moist emulsion			
NLC Deep Effect	Beate Johnen	12/2006	
- Eye Serum			Q10, highly active oligosaccharides
- Repair Cream			Q10, TiO_2, highly active oligosaccharides
- Reconstruction Cream			Q10, acetyl hexapeptide-3, micronized plant
- Reconstruction Serum			collagen, high active oligosaccharides in polysaccharide matrix
Swiss Cellular White	la prairie	1/2007	glycoprotiens, Panax ginseng root extract, Equisetum Arvense extract, Camellia Sinensis
- Illuminating Eye Essence			leaf extract, Viola Tricolor Extract
- Intensive Ampoules			
Regenerationscreme Intensiv	Scholl	6/2007	Macadamia Ternifolia seed oil, avocado oil, urea, black currant seed oil
Olivenöl	Dr. Theiss	02/2008	
Anti Falten Pflegekonzentrat			Olea Europaea Oil, panthenol, Acacia Senegal, tocopheryl acetate

Table 2-1 (Cont.)

Product name	**Producer**	**Market introduction**	**Main active ingredients**
Augenpflegebalsam			Olea Europaea Oil, Prunus Amygdalus Dulcis Oil, hydrolized milk protein, tocopheryl acetate, Rhodiola Rosea Root Extract, caffeine
Edelweiss	Audorasan	09/2008	
- Deluxe Repair Q10 Face Cream			
- Body lotion			

The scientific literatures described two types of lipid nanoparticles, that were, SLN and NLC. Three drug incorporation models are represent base on the data published by Mehnert's group. There are solid solution model, drug-enriched shell model and drug-enriched core model.

The SLN type I, the homogeneous matrix model, is composed of a homogenous matrix. The drug or active ingredient dissolved within the lipid matrix, being molecularly dispersed in the SLN core or present in form of amorphous clusters. This model is obtained when applying the hot HPH in an optimized ratio of drug and lipid, or when using the cold HPH. Being the drug incorporated, this model can show controlled release properties.

The SLN type II, the drug enriched shell model (Lukowski and Werner, 1998), shows a drug-free (or drug-reduced) lipid core covered by an outer shell of enriched with drug. This model is obtained when the drug concentration in the melted lipid is low and when applying the hot HPH. During the cooling of the homogenized NE, the lipid precipitates first, leading to a steadily increasing concentration of drug in the remaining lipid melt with increased fraction of solidified lipid. A drug-free (or drug-reduced) lipid core is formed, when the drug reaches its saturation solubility in the remaining melt, an outer shell will solidify containing both drug and lipid. Once being the drug mainly at the surface of the carrier, this model cannot show controlled release properties. Nonetheless, it can be used to obtain a burst release of drug, in association to the occlusive properties of the lipid core.

The SLN type III, the drug enriched core model (Souto and others, 2004; Westesen and others, 1997), shows a core enriched with drug which is covered by a lipid shell. It is formed when the drug concentration is relatively close to or at its

saturation solubility in the lipid melt. Under cooling of the NE the solubility of the drug will decrease, when the saturation solubility is exceeded the drug precipitates and then covered by a shell of lipid. Being the drug within the carrier and surrounded by a lipid shell, this model is useful for prolonged release purposes.

NLC can be of (i) imperfect crystal model, (ii) amorphous model, or (iii) multiple model.

The NLC type I, the imperfect crystal model, is defined by a matrix with many crystal imperfections creating vacancies where drug molecules can be accommodated in between the lipid lamellae. This model is obtained when mixing solid lipids with sufficient amounts of liquid lipids (oils), which avoid the creation of a highly ordered structure. Owing to the different chain lengths of the fatty acids and the mixture of mono-, di-, and triacylglycerols, the matrix of NLC is composed of available spaces and voids to accommodate the drug.

The NLC type II, the amorphous model, is created when mixing special lipids that do not recrystallize after cooling. Very special lipids such as hydroxyl octacosanyl hydroxyl stearate, isopropyl myristate, and dibutyl adipate, are mixed and be able to create solid particles of amorphous structure, i.e., NLC of amorphous model, which can avoid the occurrence of re-crystallization of lipid under cooling and during shelf life, minimizing drug expulsion during storage time (Müller and others, 2002b).

The NLC type III, the multiple model, comprehends very small oily nanocompartments within the solid lipid matrix of the nanoparticles by a phase-separation process. This model is created when mixing solid lipids with oils (e.g., medium and long chain triacylglycerols, oleic acid) in such a ratio that the solubility

of the oil molecules in the solid lipid is exceeded. During the cooling of the NE the lipid droplets reach the miscibility gap (40°C), the oil precipitates forming tiny oil droplets in the melted solid lipid. Subsequent solidification of the solid lipid leads to fixation of the oily nanocompartments. The advantage of this model is the increase of loading capacity for drugs that usually show higher solubility in liquid lipids than in solid lipids.

4. Technologies of production

4.1. Microemulsion based technique

This technique has been developed by Gasco (1993; 1997). A typical microemulsion composition is 10-15% lipid, 15-25% surfactant, 2-10% co-surfactant, and 50-73% water. The lipid phase is melted at approx. 60–70°C and an o/w surfactant/co-surfactant containing aqueous phase is prepared at the same temperature. When analyzing the phase diagram of such systems, the size of the microemulsion is a function of temperature, that is, the microemulsion can be converted into a different system when for example, reducing the temperature. Thus, this is why the system needs to be kept at elevated temperature during the emulsification process. Next, both lipid and aqueous phases are added and admixed to produce a microemulsion under stirring. This microemulsion is then diluted into cold water, which leads to the breaking of the microemulsion converting it into an ultra-fine NE. The reasons for breaking the microemulsion into a NE are the dilution with water and the reduction of temperature narrowing the microemulsion region. Finally, the obtained NE is cooled down which solidifies the lipid phase obtaining the lipid nanoparticles. The disadvantage of the microemulsion method is the dilution of the

particle suspension with water. Typically, the concentrations are distinctly below 1% of particle content. In the case of processing to a final dosage form, large amounts of water need to be removed, which is an inconvenient procedure. In addition, high concentrations of surfactants and co-surfactants are necessary for stabilizing the formulation but are less desirable with respect to regulatory aspects and application.

4.2. High pressure homogenizer

The first patent for SLN was filed in 1991 by Lucks and Müller (1993) describing the production of SLN by HPH. For the production of lipid nanoparticles, HPH can be performed at higher temperatures (hot HPH) or at below room temperature (cold HPH).

For the hot HPH method, the lipid is melted at approx. 5–10°C above its melting point, the drug is dissolved or finely dispersed in the melt and then this phase is dispersed by stirring in a hot surfactant solution. The obtained pre-emulsion is homogenized applying a pressure between 200 and 800 bar and 3–5 homogenization cycles in the HPH. After the homogenization, a hot NE is obtained, cooling leads to re-crystallization of the lipid followed by the formation of lipid nanoparticles.

For the cold HPH method, the lipid melt containing the drug is cooled using dry ice or liquid nitrogen, and after solidification it is ground using a mortar mill to obtain solid lipid microparticles. These particles are then dispersed in a cold aqueous surfactant solution, obtaining a pre-suspension which is further homogenized in the solid state at or below room temperature by cooling the HPH. The sheer forces and cavitation forces in the homogenizer are strong enough to break the microparticles directly into lipid nanoparticles.

4.3. Ultra-sonication

This technique described by Ricci *et al.* (2005). Lipid was melted at 80ºC and oil and active compound were added. The melted lipid phase was dispersed in the hot (80ºC) surfactant solution by using a high speed stirrer (Ultra TurraxT25, IKA-Werke GmbH & Co.KG, Staufen, Germany) at 8000 rpm. The obtained pre-emulsion was ultrasonified by using a UP 400 S (Ultraschallprozessor, Dr. Hielscher GmbH, Germany) maintaining the temperature at least 5ºC above the lipid melting point. After ultrasonication, the obtained dispersion was cooled in an ice bath in order to solidify the lipid matrix and to form lipid nanoparticle. A great advantage of this method is the fact that the equipment is common in every lab and the production can easily be done. The problem of high speed stirring was a broader particle size distribution ranging into the micrometer range. This leads to physical instabilities such as particle growth upon storage. This could be improved by higher surfactant concentrations, which in order might be correlated with toxicological problems after parenteral administration. A further disadvantage is potential metal contamination due to ultrasonication (Wissing and others, 2004).

4.4. The solvent emulsification evaporation method

The solvent emulsification evaporation technique was described by Sjostrom and Bergenstahl (1992). In this technique the lipid is dissolved in an organic solvent nonmiscible with water, such as methylene chloride, cyclohexane, or chloroform. The drug/active ingredient is dissolved or dispersed in this phase (Sjostrom and others, 1993a; 1993b). This organic phase is emulsified by mechanic stirrer in an aqueous solution containing an o/w surfactant to produce an o/w emulsion. The organic

solvent is further removed by evaporation under stirring or reduced pressure. Upon evaporation, a nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium.

4.5. The solvent displacement method

This technique described by Fessi *et al.* (1992). In the solvent displacement technique, the lipid material is dissolved in a semi-polar water-miscible solvent, such as EtOH, acetone, or MeOH, where the drug/active ingredient is also dissolved or dispersed. An o/w surfactant containing aqueous phase is also prepared. The organic phase is injected into the aqueous phase under magnetic stirring. A violent spreading is observed because of the miscibility of both phases. Droplets of solvent of nanometer size are formed from the o/w interface. These droplets are rapidly stabilized by the surfactant molecules being in the aqueous phase, until diffusion of the solvent is complete and lipid precipitation has occurred. Removal of solvent can be performed by distillation, ultrafiltration or reduced pressure, and lipid nanoparticles are formed after total evaporation of the water-miscible organic solvent.

4.6. The emulsification diffusion method

The emulsification diffusion technique was developed by Quintanar-Gerrero *et al.* (1999). In the emulsification diffusion technique, partially water soluble solvents are used, such as benzyl alcohol or THF. The solvent is first saturated with water to ensure the initial thermodynamic equilibrium between those two liquids (water and solvent). The lipid is dissolved in the saturated solvent producing an organic phase where the drug/active ingredient is added. This organic phase is emulsified, under

vigorous stirring, in an aqueous solution containing the emulsifying agent obtaining an o/w emulsion. Adding water to the system under moderate mechanical stirring, causes solvent diffusion into the external phase and the lipid starts precipitating. The organic solvent can be afterwards removed by distillation or ultra-filtration. After the organic solvent being totally removed, an aqueous dispersion of lipid nanoparticles is formed.

4.7. The phase inversion based method

The phase inversion based method has been described by Heurtault *et al.* (2000; 2002). This method is based on a two-step process involving temperature cycling. First, all components are placed on a magnetic stirrer using a temperature program from room temperature to for example 85°C, which is then followed by progressive cooling to 60°C. Three temperature cycles (85–60–85–60–85°C) are applied to reach the inversion process defined by the temperature range. In a second step, an irreversible shock is induced by dilution with cold water. This fast cooling diluting process leads to the formation of stable nanoparticles under cooling.

5. Antioxidant

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidative metabolism is essential for the survival of cells (Antolovich and others, 2002). However, oxidation reactions can produce free radicals, which start chain reactions that damage cells. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They can terminate chain reactions by removing free radical intermediates, and inhibit other oxidation

reactions by being oxidized themselves. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). Water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma. Antioxidants in this group are ascorbic acid (vitamin C), glutathione, lipoic acid, uric acid, etc. Lipid-soluble antioxidants protect cell membranes from lipid peroxidation. Antioxidants in this group are carotenes, α-tocopherol (vitamin E), ubiquinol (coenzyme Q10), etc. On the other hand, antioxidants are classified into 3 groups by their activities. The first group inhibits the oxidation reaction. Antioxidants in this group are tocopherol, BHA, BHT, NDGA, alkyl gallates. The second group is represented as reducing agents (a substance with itself readily oxidized). Antioxidants in this group are thiols, ascorbic acid and polyphenols. The last group is represented as sequestering, or chelating agent (defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell.

5.1. Lycopene

Lycopene, sometimes called rhodopurpurin, is a phytochemical, synthesized by plants and microorganisms but not by animals. Human are unable to synthesize carotenoids *de novo*, we can obtain them from dietary food. Normally, carotenoid levels in human skin are in range of 0.2-0.6 nmol/g wet tissue (without subcutaneous fat). Lycopene is accumulated in most human tissues such as skin, breast, adrenal, liver, testis. Tissue distribution of dietary carotenoids including lycopene is not uniform. Tissue specific carotenoid distribution suggests that certain carotenoids may exert a unique biological effect in some tissues but not in the others. Table 2-2 shows the lycopene levels in human tissues reported by different investigators.

Table 2-2 Lycopene levels in human tissues.

Tissue	Lycopene (nmol/g wet wt)	Reference
Testes	4.34 - 21.36	Kaplan and others, 1990
Adrenal	1.90 - 21.60	Kaplan and others, 1990
Prostate	0.80	Kaplan and others, 1990
Liver	1.28 - 5.72	Kaplan and others, 1990
Breast	0.78	Nierenberg and Nann, 1992
Pancreas	0.70	Nierenberg and Nann, 1992
Lung	0.22 - 0.57	Nierenberg and Nann, 1992
Kidney	0.15 - 0.62	Schmitz and others, 1991
Colon	0.31	Schmitz and others, 1991
Skin	0.42	Schmitz and others, 1991
Ovary	0.30	Stahl and others, 1992
Stomach	0.20	Stahl and others, 1992
Brainstem	Not detectable	Stahl and others, 1992

It is note that lycopene was found to concentrate and be the most prominent carotenoid in adrenal glands, testes, liver and prostate. The exact biochemical mechanism for the high concentration in these tissues is not clear. One hypothesis is that these tissues have large number of lipoprotein receptors and lycopene is mainly transported through lipoprotein. This potential natural antioxidant and radical scavenger found in tomatoes and tomato-based food products, watermelon, and pink grapefruit (Barba and others, 2006; Choudhari and others, 2009; Stahl and others, 2006) has so far become one of the most interesting active molecules. It functions as

anti-inflammatory, anti-cancer, and anti-mutagenic agent and exhibits a high physical quenching rate constant for singlet oxygen *in vitro* (Giovannucci, 1999; Heber and Lu, 2002; Oppen-Bezalel, 2008; Shi and others, 2003). Hence, it can decrease the risks of cancer of prostate, lung, stomach, pancreas, colon, rectum, esophagus, oral cavity, breast (Rao and Agarwal, 1999; Stahl and Sies, 1996; Wenli and others, 2001). Lycopene also has an anti-stress effect related to inhibition of the harmful effects from UV exposure, because it is an effective agent against solar stress. The compound protects against premature aging and age-related disorders due to oxidative damage and stress. Radioprotective as well as antibacterial activity of lycopene were established almost 40 years ago using laboratory mice. Dietary lycopene increased the survival rate of mice exposed to whole body X-irradiation. Intraperitonial injections of lycopene also protected the mice from bacterial infections and inhibited the development of ascites tumors. The safety aspect of bioactive compounds in products has also been received much attention from scientists to avoid any side effects. Either synthetic lycopene or from natural sources have been reported to be safe as GRAS status when used in as food additive (Kong and others, 2010; Trumbo, 2005). The molecular weight of lycopene is 536.85 Daltons with the general structure being an unsaturated acyclic carotenoid ($C_{40}H_{56}$) with 11 linear conjugated and two non-conjugated double bonds (Figure 2-7), which imparts a red coloration as well as fat and lipid soluble characteristics. It is not the precursor for vitamin A, since it lacks the terminal β-ionic ring found in the basic structure of vitamin A.

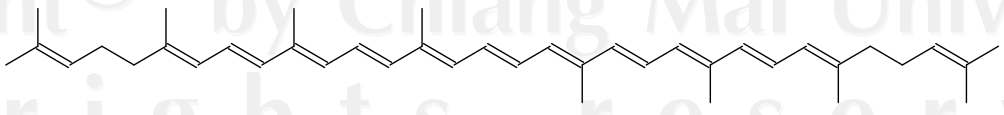

Figure 2-7 All-trans lycopene.

As a result of the 11 conjugated carbon-carbon double bonds in its backbone, lycopene can theoretically assume 2^{11} or 2048 geometrical configurations (Zechemister and others, 1943). A large number of geometrical isomers are theoretically possible for all-trans lycopene, and this is independent of its thermodynamic stability. According to Pauling (1939) and Zechmeister *et al.* (1941) only certain ethylenic groups of a lycopene molecule can participate in cis-trans isomerization because of steric hindrance. Lycopene's chain containing seven double bonds that can be isomerized to mono-cis or poly-cis due to the exposure to high temperatures, light, oxygen, acids, catalyst and metal ions (such as Cu^{2+}, Fe^{3+}) (Shi and others, 2002). In fact, only about 72 lycopene cis-isomers are structurally favorable (Zechemister, 1962). All-trans, 5-cis, 9-cis, 13-cis, and 15-cis are the most commonly identified isomeric forms of lycopene with the stability sequence being 5-cis > all-trans > 9-cis > 13-cis > 15-cis > 7-cis > 11-cis (Chasse and others, 2001a; 2001b), so that the 5-cis form is thermodynamically more stable than the all-trans isomer. Figure 2-8 illustrates the structural distinctions of most common lycopene geometrical isomers.

Although lycopene oxidation and isomerization of all-trans form to cis-form can be induced by light and heat, it was reported by Shi *et al.* (2003) and Thompson *et al.* (2000) that lycopene was relatively stable if heated at temperatures below 100ºC, the tomatoes' lycopene content was not affected by cooking for 4, 8, and 16 min at 100ºC. Although it is generally recognized that heat treatment of tomatoes induces the formation of cis-isomers from the all-trans form present in raw tomatoes, specific types of isomers formed and their biological significance is not fully understood. Bioavailability of lycopene is influence not only by its isomeric form but also the

presence of other dietary components including dietary fat, other carotenoids, vitamins and minerals (Jain and others, 1999).

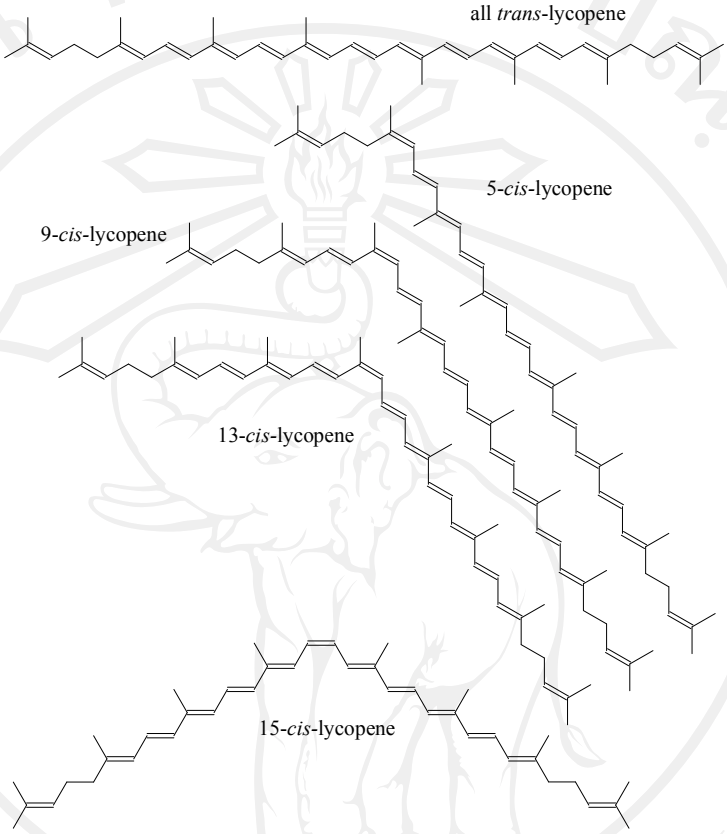

Figure 2-8 Structures of most common lycopene isomers.

Even if all-trans conformation is found in nature, human tissues absorb mainly cis-isomers. This is due to the shorter length and the greater solubility of cis-isomers in mixed micelles. Ferret studies show the result that cis-isomers are more bioavailable than all-trans conformation (Boileau and others, 2002). From the *in vitro* studies, cis-isomers are more soluble in bile acid micelles. They may be preferentially absorbed into chylomicrons. Cis-isomers of lycopene have physical characteristics and chemical behaviors distinct from those of their all-trans counterpart, including decreased color intensity and lower melting points; they are more polar than their all-trans counterparts, less prone to crystallization, and more soluble in oil and

hydrocarbon solvents. Experimental results revealed that cis-isomers of lycopene are better absorbed by human than the all-trans form (Xianquan and others, 2005). Furthermore, an *in vitro* study of Failla *et al.* (2008) using Caco-2 cells showed that the uptake of cis-lycopene was significantly greater than for all-trans isomer. Thus, cis-isomers have higher bioavailability than all-trans isomers. In human plasma, lycopene is an isomeric mixture, containing at least 60% of the total lycopene as cis-isomers (Schierle and others, 1997). As a fat soluble compound, lycopene has a similar absorption as dietary fat. In the stomach and duodenum, lycopene will separate from the food matrix and subsequently dissolve in the lipid phase (Krinsky and Johnson, 2005). Prior to absorption, the lipid phase will form droplets, resulting from the reaction with bile salts and pancreatic lipases. Then, it enters the duodenum and appears as the multi-lamellar lipid vesicles (Clinton, 1998). Finally, the lipid vesicles will absorb into small intestine (intestinal mucosal lining) *via* passive or diffusion process. Additionally, there are *in vitro* studies suggested that the intestinal absorption of lycopene was aided by the participation of a specific epithelial transporter (During and Harrison, 2004; 2005). They are incorporated into chylomicrons and released into lymphatic system for transport to the liver. Lycopene are transported by the lipoproteins into the plasma for distribution to the different organs. It is the most predominant carotenoid in human plasma and has a half life of about 2-3 days (Rao and Agarwal, 1999). Very little is known about *in vivo* metabolism of lycopene. Only a few metabolites, such as 5,6-dihydroxy-5,6-dihydro lycopene, have been detected recently in human plasma. It was suggested that lycopene may undergo *in vivo* oxidation to form epoxides which then may be converted to 5,6-dihydroxy-5,6-dihydro lycopene through metabolic reduction. Owing

to its lipophilic nature lycopene was found to concentrate in LDL and VLDL fractions and not in HDL fraction of the serum.

So far, it was summarized by Bramley (2000) that lycopene was more interesting than β-carotene. Lycopene possesses the highest TEAC value among common carotenoid compounds. Erdman *et al.* (2009) reported that there was almost universal agreement that lycopene was an excellent *in vitro* antioxidant, especially in quenching singlet oxygen. Lycopene, because of its high number of conjugated dienes, is the most potent singlet oxygen quencher among natural carotenoids (Table 2-3).

Table 2-3 Comparison of antioxidant activities of carotenoids: singlet oxygen quenching.

Lycopene	
Singlet oxygen quenching, $10^9 \times Kq$ ($m^{-1}.s^{-1}$)	31
Radical scavenging (Trolox equivalents)	2.9
Reaction of carotenoid radical anions with O_2, $10^8 \times k$ ($m^{-1}.s^{-1}$)	2
Other carotenoids' singlet oxygen quenching, $10^9 \times Kq$ ($m^{-1}.s^{-1}$)	
γ-carotene	25
α-carotene	19
β-carotene	14
Lutein	8
Astaxanthin	24
Bixin	14
Canthaxanthin	21
Zeaxanthin	10

The quenching activity of carotenoid species depends on the number of conjugated double bonds and is influenced to a lesser extent by carotenoid end groups or the nature of substituents in carotenoids containing cyclic end groups. Lycopene was also reported to inactivate hydrogen peroxide and nitrogen dioxide. Mortensen *et al.* (2001) using pulse radiolysis technique demonstrated carotenoids' ability to scavenge nitrogen dioxide ($NO_2^{\bullet}$), thiyl ($RS^{\bullet}$), and sulphonyl ($RSO_2^{\bullet}$) radicals. Lycopene was found to be at least twice as active as β-carotene in protecting lymphocytes against $NO_2^{\bullet}$ radical induced membrane damage and cell death. There are indications that lycopene is the most potent scavenger of ROS among other major dietary carotenoids. Lycopene protected human LDL against photosensitized oxidative damage. Skin lycopene was destroyed preferentially over β-carotene during UV light exposure in humans suggesting a superior role of lycopene over β-carotene in mitigating oxidative damage in tissues. When lycopene reacts with the superoxide radical ($O_2^{\bullet-}$) electron transfer occurs with the formation of the anion radical, as depicted in reaction.

$$\text{Lycopene} + O_2^{\bullet-} \longleftrightarrow \text{Lycopene}^{\bullet-} + O_2$$

Yeh and Hu (2000) studied on prooxidant effects of lycopene *in vitro* and demonstrated that lycopene could be either an antioxidant or a prooxidant depending on the oxidants used. Stahl and Sies (2004) summarized on their study that lycopene might act as a prooxidant *in vitro*, depending on experimental conditions. They found the optimal protection level of lycopene at 0.05 nmol/mg protein in human fibroblasts. Further increasing of lycopene levels in cells led to prooxidant effects measured by TBARS assay. Anguelova and Warthesen (2000) reviewed that lycopene (20μg/g oil) alone could act as a prooxidant, while the combination of lutein or lycopene with γ-tocopherol showed an antioxidant activity. However, there is no evidence to support

the hypothesis that carotenoids can act as prooxidants within the highly complex biological system (Arab and others, 2001; Astley and others, 2004; Edge and others, 1997; Erdman and others, 2009; Haseen and others, 2009; Porrini and Riso, 2000; Stahl and Sies, 2003; Trumbo, 2005; Young and Lowe, 2001).

Baldermann *et al.* (2008) characterized the partition coefficient of lycopene *in vitro*, they found the concentration ratio of this compound between the stationary and mobile phase that it was 0.96 at 10ºC, 1.01 at 15ºC, and 1.15 at 20ºC. In their experiment, mobile phase, which is in the upper, consists of hexane : CH_2Cl_2 : ACN = 30 : 11 : 18 v/v/v. Stationary phase, which is lower polar ACN rich phase, is below.

6. Lipids

The lipids used in this work are well tolerated, of GRAS status, accepted for human use and they are also *in vivo* biodegradable.

6.1. Solid lipids

6.1.1. Orange wax

Orange wax is a natural plant wax which is extracted from the peels of the *Citrus* (orange) fruit. Orange wax consists mainly of hydroxy-monoesters and unsaturated monoesters (neutral lipids) of unsaturated fatty acids and long-chain alcohols. The free fatty acid (C12-C26) portion consists of linoleic, oleic, linolenic, arachidic, and erucic acids.The fatty alcohol portion of the ester is predominantly dotriacontanol (C32) and tetratriacontanol (C34). Moreover, orange wax also contains hydrocarbons (C21-C33), sterol esters, free sterols, glycolipids, phospholipids, carotenoids, and flavonoids (Puleo and Peters, 1994). The primary chemical compositions of orange wax by percent are presented in Table 2-4.

Table 2-4 Primary chemical composition of orange wax by percent.

Components	Percent(%)
Unsaturated-monoesters, hydroxy-monoesters,and monoesters	50 to 65
Free fatty acid(C12 to C26)	6 to 15
Hydrocarbons(C21 to C33)	8 to 15
Sterol esters	5 to 18
Free sterols	4 to 8
Free alcohols	2 to 7
Carotenoids	0.5 to 2
Glycolipids	0.5 to 2
Phospholipids	0.5 to 2
Flavonoids	0.2 to 1
Fragrance compounds(natural)	1.1 to 0.8

In general, skin lipids comprise polar lipids, neutral lipids, and sphingolipids. All of these general chemical groups are represented in orange wax. There are striking similarities in the chemical compositions of orange wax and skin lipids in both chemical functionality and component weight percent distribution as shown in Table 2-5.

Table 2-5 Skin lipid composition compared to orange wax chemistry.

Lipid type	Human skin (various sites)	Orange wax
Wax esters	15 to 25%	50 to 65%
Sterol esters	2 to 6%	5 to 18%
Triglycerides and complex esters	12 to 25%	
Free sterols	2 to 10%	4 to 8%
Free fatty acids	2 to 20%	6 to 15%
Hydrocarbons/alkanes	3 to 6%	8 to 15%
Squalene	3 to 10%	
Glycosphingolipids I and II	2 to 7%	0.5 to 2%
Ceramides	10 to 22%	
Phospholipids	3 to 6%	0.5 to 2%
Cholesterol sulfates	1 to 6%	

Orange wax carries with the properties of UVA and UVB sunscreen-enhancing (Reynhardt and Riederer, 1991), antioxidant (Kolesnik and others, 1989; Puleo and Rit, 1992), antimicrobial (Caccioni and others, 1998; Ndagijimana and others, 2004), and anti-inflammatory (Puleo and Peters, 1994). It can be used as an emollient and helpful for refreshing and moisturizing. All of these multifunctional advantages of using orange wax are based on its chemistry. The acid value of this lipid is 18.59, congealing point is 53.8°C, saponification value is 97.87, and the hydroxy value is 12.5 (Orange wax COA, 2005). Orange wax is also biodegradable, green product, derived from a renewable plant source, and acceptable for cosmetic use in the United States and Europe, and has been submitted for approval as a cosmetic ingredient in Japan. Therefore, orange wax could be classified as a skin friendly lipid material and especially pick up in this study as the main solid lipid ingredient.

6.1.2. Cholesterol

High cholesterol has been linked to causing heart disease, but are there any benefits of cholesterol? The truth is cholesterol is a 100% necessary ingredient in the processes that sustain life. Yet few people know about its benefits, and even medical experts are still discovering the many roles that it plays. Cholesterol is a chemical compound that is naturally produced by the body and is structurally a combination of lipid (fat) and steroid (Figure 2-9). It is one of three major lipid classes found in the skin (Bouwstra and others, 2001) that serve essential functions not only in terms of good skin health but also the health of the entire body (Norlen and Engblom, 2000). Cholesterol is a building block for cell membranes and exists in the outer layer of every cell in our body and has many crucial functions. Our bodies need cholesterol to

maintain healthy cell walls (Kirjavainen and others, 1996). It modulates membrane fluidity over the range of physiological temperatures. The skin barrier is assaulted frequently in daily life by hot water, detergents, solvents, mechanical trauma, and occupation-related chemicals. If these insults are frequently repeatedly and/or insufficiently repaired, they threaten the organism with desiccation due to accelerated TEWL. In addition, lipid levels may also become abnormal because of changes that occur with aging, various disorders (including some hereditary ones), use of certain drugs, or lifestyle (such as consuming a high-fat diet, being physically inactive, or being overweight) (Waller and Maibach, 2006). If cholesterol is depleted, the skin cells begin to deteriorate and crumble away. The outer level of the skin, known as the SC, begins to flake. The result is dry skin or so-called xerosis (Harding and others, 2000). This means not simply skin that only lacks water but also be dysfunctional skin where there is an accumulation of attached corneocytes at the skin surface. The skin feels rough, tight, looks dull because light is scattered by the uneven surface, looks pale because the pinky glow from the microcirculation is obscured, may show visible scaling and is susceptible to irritation, and may crack in regions subject to stretching forces (e.g. knuckles). To avoid this, cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3-5.0%w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity (Rowe and others, 2009). Topical application of cholesterol effectively soothes and plumps up dry skin (Proksch and Lachapelle, 2005). Since this, cholesterol is interesting to use in the development of NLC.

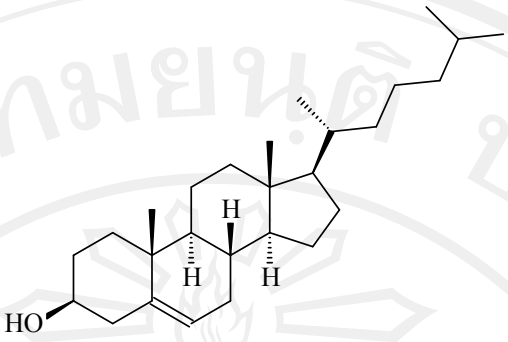

Figure 2-9 The chemical structure of cholesterol.

6.1.3. *Mangifera indica* seeds butter

Mango (*Mangifera indica* L.), which belongs to the family *Anacardiaceae*, is the most cultivated fruit in Thailand. The mangos' popularity is on the rise due to its high nutraceutical and pharmaceutical value. The mango is unique because each of its parts: fruit, pulp, peel, seed, leaves, flowers and the bark are utilizable (Masibo and He, 2009). Processed mango products are among the major goods exported from Thailand. Therefore, several million tons of mango seed wastes are produced annually from factories. The fat from mango seeds has attracted the attention of scientists in recent years as a cocoa butter substitute, because the former has a fatty acid and triglyceride profile similar to that of cocoa butter (Baliga and Shitole, 1981; Hemavathy and others, 1987). Mango butter is a polymorphous natural fat formed consists of a mixture of several compounds mainly oleic acid, palmitic acid, linoleic acid, and stearic acid (Mango butter MSDS, 2008). The saponification value of this fat is 197, and the iodine value is 48.6 wijs. The HLB value is about 8 and a specific gravity is 0.89 at 50°C (Mango butter COA, 2008). A peroxide value is lower than 6 meq O_2/kg indicates a high chemical stability of fat (Hommoss, 2008; Souto and others, 2006). Mango butter has a peroxide value 1.70 meq/kg, thereby, it is

considered to be stable fat. Recently, mango butter was developed as a topical cream for effective dermatological care. Mango fats show excellent bioactive properties like wound healing, antiseptic, bactericidal and bacteriostatic, antimicrobial, and anti-inflammatory activities (Dharkar, 2011).

6.1.4. Beeswax

Beeswax is defined according to the European Pharmacopoeia. Bees secrete wax from four pairs of special glands, called wax glands, on the underside of their abdomens (Kameda, 2004). Beeswax, with its unique characteristics, is being used in the development of new products in various fields such as cosmetics (Koga, 2000), pharmacy (Al-Waili, 2003), foods, pharmaceuticals (Dorset, 1999), candle making, art, engineering (Mariya and Nikolay, 2002), industry and for many other purposes. Al-Waili (2005b) reported that honey mixture containing raw honey, olive oil and beeswax inhibited growth of *C. albicans* or *S. aureus*. Honey mixture appears useful in the management of dermatitis and psoriasis vulgaris (Al-Waili, 2003). This topical treatment was safe and well-tolerated, and also demonstrated clinical and mycological benefits in the treatment of diaper dermatitis (Al-Waili, 2005a). Beeswax is a hard wax formed and consists of policosanol (Irmak and others, 2006) and long chain carbon components including alkanes that contain 21–33 carbon atoms, alcohols, free acids that contain 22–30 carbons, and esters that contain 40–52 carbons as well as other materials (Garnier and others, 2002; Kimpe and others, 2002). Beeswax is also known to contain long-chain diesters (Tulloch and Hoffman, 1972). The acid value of this wax is range from 17-24, and the iodine value is range from 72-79.

6.2. Liquid lipids

6.2.1. Rice bran oil

Rice bran oil (*Oryza Sativa*) or so-called rice oil is obtained during the process of milling the rice (Most and others, 2005) and comes from the bran of the rice kernel which is the part containing the most oil. It is considered by some to be the "Worlds healthiest edible oil". The main components of rice bran oil include unsaturated fatty acids, triterpene alcohols, phytosterols, tocotrienols, alpha-tocopherol, gamma-oryzanol, squalene, and nutrients (Orthoefer, 2005; Sugano and Tsuji, 1997). These components are useful in protecting the body's cells against the effects of free radicals and aid in slowing down the effects of aging, and thus, slowing the formation of facial wrinkles (Santa-María and others, 2010). The gamma-oryzanol impedes the progress of melanin pigmentation and is effective in keeping skin smooth (Lerma-García and others, 2009). Squalene is thought to help support the collagen within the skin. In addition, it also contains proanthrocyanidins which protect collagen and elastin which in turn play in a large part in maintaining the suppleness of the skin. As rice bran oil is particularly high in fatty acids (oleic, 46 percent; linoleic, 36 percent; and linolenic, 1 percent), it is very beneficial for mature, delicate and sensitive skin. Therefore, it is commonly used in cosmetics. Rice bran oil does not require hydrogenation for stability. The shelf life of rice bran oil is very good which helps to translate to a long shelf life for the products made from it. And due to a small molecule of rice bran oil, this makes it easier to penetrate the skin without being greasy. It is light texture that absorbs quickly into the skin. At the same time it makes the skin soft, moisturized and nourished. It also has been used in Japan by women for centuries to help smooth out wrinkles as well as to provide a slight amount of sunscreen protection. It is known for

its spreadability, hence it was used in lipsticks and creams. Besides, when rice bran oil is uptaken by the body, it has demonstrated an ability to improve the plasma lipid profiles in animal and human studies, reducing total plasma cholesterol and triglyceride concentration and increasing the high density lipoprotein cholesterol level. Other potential benefits of rice bran oil and gamma-oryzanol include modulation of pituitary secretion, inhibition of gastric acid secretion, antioxidant action and inhibition of platelet aggregation. For a long time, studies have shown that rice bran oil can help fight diseases, enhance the immune system, fight free radicals and more.

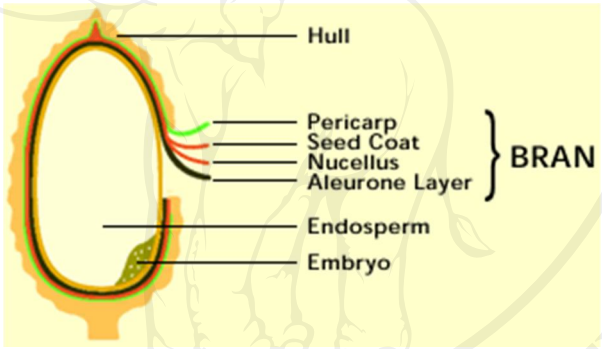

Figure 2-10 The structure of the rice kernel. The bran fraction, which includes the germ or embryo in most commercial milling operations, represents only about 8% of paddy weight but contains about three-fourths of the total oil (http://www.ricebranoil.info/index.html, available 15/10/2011).

Rice bran oil has a light yellow color and a mild natural aroma, almost odorless. It is notable for its very high smoke point of 490°F (254°C) and its mild flavor, making it suitable for high-temperature cooking methods. The high smoke point prevents fatty acid breakdown at high temperatures, which means that even in the hottest of situations rice bran oil won't smoke or breakdown. Your foods will be

taste better, and they will be less likely to stick to the grill or griddle. Moreover, consumed at room temperature or cooler, rice bran oil is rich in vitamin E, gamma-oryzanol (an antioxidant, a group of ferulate esters of triterpene alcohols and phytosterols, that is used for many alternative herbal therapies, and may help prevent heart attacks) (Duve and White, 1991), and phytosterols (compounds believed to help lower cholesterol absorption) (Nicolosi and others, 1993; Rukmini and Raghuram, 1991), which may provide associated health benefits. Rice bran oil contains a range of fats, with 47% of its fats monounsaturated, 33% polyunsaturated, and 20% saturated. The fatty acid composition of rice bran oil is summarized in Table 2-6.

Table 2-6 The fatty acid composition and their percentage of rice bran oil.

Fatty acid	Percentage
Palmitic	15.00%
Stearic	1.90%
Oleic	42.50%
Linoleic	39.10%
Linolenic	1.10%
Arachidic	0.50%
Behenic	0.20%

6.2.2. Pomegranate seeds oil

The pomegranate (*Punica granatum* L.) seeds oil is a highly celebrated medicinal food plant chosen as the symbol of medicine for the 2000 UK Millennial Festival of Medicine (Langley, 2000). Current interest focuses largely on the pomegranate's antioxidant and anti-inflammatory activities (Aviram and others, 2000; Gil and others, 2000; Schubert and others, 1999; Singh and others, 2002). If assumed pomegranate seeds oil through diet, it can improve immune function, reduce hepatic

triacylglycerol accumulation and act as a chemo-preventive agent against hormone-related human cancers (prostate, breast) (de Nigris and others, 2007; Kim and others, 2002; Kohno and others, 2004; Lansky and others, 2005; Saito and others, 2008). Pomegranate seeds oil has several features that make it an attractive nutraceutical ingredient as a consequence of its novelty, good acceptance by the consumers, cheap availability and promising phytochemical composition. Pomegranate seeds oil comprises 10–20% of total seed weight, with an ideal highly punicic acid (Figure 2-11) level up to 75-85%, which activities against inflammation and metabolic syndrome have been confirmed by various experiments (Lansky and Newman, 2007; Mukherjee and Bhattacharyya, 2006; Yamasaki and others, 2006). Pomegranate seeds oil can be considered an interesting alimentary source of substances of nutraceutical value involved in the modulation of cholesterol metabolism (Caligiani and others, 2010). It was also shown to stimulate keratinocyte proliferation in monolayer culture, therefore promoting regeneration of epidermis (Aslam and others, 2006). Pomegranate seeds oil was purchased from Guangzhou Herbs-Ex Inc. (Guangzhou, China). The oil is 100% from natural extracted and purified by supercritical CO_2 extraction from dried seeds of *Punica granatum* L. It has a clear and golden yellow with a light characteristic odor of pomegranate seeds. Pomegranate seeds oil is insoluble in water and miscible with fatty oils and fat-soluble organic solvents (Pomegranate seeds oil MSDS, 2010). The specific gravity of oil is between 0.9390 and 0.9590. The refractive index is between 1.5150 and 1.5250. The acid value is less than 10 mg KOH/g and a peroxide value is less than 10 meq/kg (Pomegranate seeds oil COA, 2010).

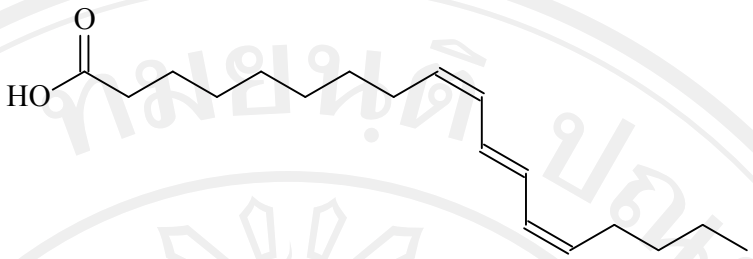

Figure 2-11 The chemical structure of punicic acid.

6.3. Emulsifiers

In order to keep emulsions stable over an extended period and to prevent separation of the phases, so-called emulsifiers are added to the emulsions. The emulsifiers are generally molecules with a polar, hydrophilic structural element and a nonpolar, lipophilic structural element.

6.3.1. Eumulgin™ SG

Eumulgin™ SG, a strong anionic and skin friendly emulsifier based on sodium stearoyl glutamate (Cognis corporation, 2009). It has high efficiency emulsification tolerating electrolytes. The emulsification is possible with low concentration of 0.25%, the recommended concentration goes up to 1.0%. The outstanding compatibility with organic and inorganic electrolytes is superior to other emulsifiers in the market. A further benefit, especially in comparison to sodium stearate, is the possibility of application over a wide pH-range, including lower value of pH 5. Eumulgin™ SG is suitable not only for sun care concepts with water-soluble UV filters but also for face and body care concepts containing high levels of actives. Previously, Eumulgin™ SG was used in many cosmetic and cosmeceutical products such as sunscreen formulation, personal skin care (Ansmann and others, 2012), cleaning and care of skin and hair (Eisfeld and others, 2007), topical regenerate skin

tissue (Guasti, 2010). Eumulgin™ SG is also used as a composition for coating the eyelashes such as mascaras and the field of making up or caring for the eyelashes (Stephane, 2008), as a composition in a cosmetic emulsion (Tesch and others, 2011).

6.3.2. Tegocare™ 450

Chemically Tegocare™ 450 is stearyl glucoside. The INCI name is polyglyceryl 3 methyl glucose distearate. It is a non-ionic and a PEG-free emulsifier based on natural renewable raw material. It is suitable for the formation of o/w creams and lotions (Tegocare™ 450 product data sheet, 2008). It can also be used in sunscreen formulations (Wissing and Müller, 2002). The HLB of this emulsifier is approximately 12 and exists as solid pellets form with ivory color and the slight typical odor (Tegocare™ 450 MSDS, 2008). Tegocare™ 450 has a melting point between 52.0°C and 58°C. The acid value is less than 12 mg KOH/g, the saponification value is between 120.0 mg KOH/g and 140.0 mg KOH/g. The iodine value is less than 5.0 gI/100g (Tegocare™ 450 COA, 2008). It is insoluble in water, but it is approximately 100g/L emulsifiable at 60°C. Previously, Tegocare™ 450 (1.8-3.6%) had been used in lipid nanoparticle preparation containing 20-30% lipid phase (Souto and others, 2005b; Wissing and Müller, 2001). Tegocare™ 450 was also used to stabilize many drugs and active compounds such as benzochinon derivate (Dingler and Gohla, 2002), ascorbyl palmitate (Teeranachaideekul and others, 2007a), retinol (Jenning and others, 2002) and coenzyme Q10 (Obeidat and others, 2010) in preparation of lipid nanoparticle.

6.3.3. Inutec™ SP1

The INCI name is inulin lauryl carbamate, and also known in Japanese name as carbamic acid lauryl inulin. It is fine white powder. Chemically it is hydrophobized

inulin (Inutec™ SP1 MSDS, 2007). Inutec™ SP1, is a non-ionic and a polymeric EO-free emulsifier, obtained by grafting linear polyfructose (inulin) with hydrophobic lauryl chains by the reaction between isocyanates and the polyfructose backbone in the presence of a basic catalyst such as a tertiary amine or a Lewis acid (Stevens and others, 2001a; 2001b). In this way alkyl groups are introduced which are randomly distributed on the polysaccharide backbone. The structure of Inutec™ SP1 is depicted in Figure 2-12. The resulting inulin carbamates possess tensioactive properties and can be used as an emulsifier for o/w emulsions in pharmaceutical formulations, as a dispersant for hydrophobic particles and in the production of foams. With Inutec™ SP1, it is possible to prepare stable cosmetic emulsions, form sprayables to butter-like viscosity creams with a light, non-sticky and neutral skin feel. Inutec™ SP1 has a low influence on surface and interfacial tension compared to so-called tension-actives. Therefore, it does not behave as a foaming or wetting agent, nor does it show a clear CMC. It plays the important role in stabilization of hydrophobic particles and oil droplets against flocculation and/or coalescence *via* a mechanism called steric stabilization. The alkyl groups of emulsifier are strongly adsorbed on an oil droplet, while the inulin chain is the stabilizing part of the molecule, forming strongly hydrated loops dangling in the external phase (Inutec™ SP1 product data sheet, 2007; Booten and Levecke, 2003). Other advantages of Inutec™ SP1 are a low viscosity and preservation of its stabilizing effect on emulsions and suspensions with high electrolyte concentrations. Inutec™ SP1 is also reported to use as a carrier in the formulation for poorly soluble drugs (Janssens and others, 2008; Mooter and others, 2006).

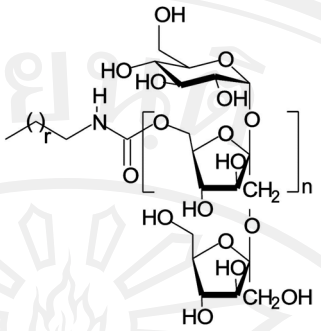

Figure 2-12 The chemical structure of hydrophobized inulin.

6.3.4. Plantacare™ 1200

The INCI name is lauryl glucoside. Plantacare™ 1200 is a cloudy, viscous, aqueous solution of a C12-C16 fatty alcohol polyglycoside. The HLB value is approximately 16-17. The turbidity of the product is attributable to a combination of its magnesium oxide content (max. 600 ppm magnesium) and the pH value at which it is supplied. This turbidity has no negative effects on the products properties and disappears if the pH value is adjusted to below 7. Plantacare™ 1200 is a non-ionic and non-ethoxylated surfactant with good dermatological compatibility, biodegradable, excellent skin and environmental tolerability, and viscosity enhancing effects (Goebel and others, 2010). Therefore, it is suitable for using as an additive or a co-surfactant in cosmetic surfactant cleansing preparations as it is already used in several cleaning and skincare products (Andree and Middelhauve, 1991; Steber and others, 1995). If the product is stored at temperatures below 38°C crystallization may occur. Depending on the storage time sedimentation may occur. Therefore, the product should be heated and stirred until uniform before use. The density is between 1.07 g/cm³ and 1.08 g/cm³ at 40°C. Plantacare™ 1200 has a high pH-value and for this reason the product contains no preservatives (Plantacare™ 1200 product data sheet, 2011).

6.3.5. Sucrose ester emulsifiers

They are products of Surfhop^{TM} SE Cosme marketed by Mitsubishi-Kagaku Foods Corporation (Japan). Sucrose esters are non-ionic emulsifiers with many applications widely used in the food, cosmetic and personal care industries and there has recently been great interest in their applicability in different pharmaceutical fields. They are natural, low toxicity, biocompatibility and excellent biodegradable excipients with well-known emulsifying and solubilizing behavior (Youan and others, 2003). Sucrose stearate improves the stability of the emulsions (Franco and others, 1995). Currently the most common pharmaceutical applications of sucrose esters are for the enhancement of drug dissolution and drug absorption/permeation, and in controlled-release systems (Szűts and Szabó-Révész, 2012). Sucrose ester emulsifiers have a wide HLB range from 0 to 16. The HLB is controlled by level of esterification and fatty acid type (Sucrose ester emulsifiers product data sheet, 2005). The examples of sucrose ester emulsifiers including their properties are summarized in Table 2-7.

Table 2-7 Summary of composition and HLB of sucrose ester emulsifiers.

Sucrose ester	Type	HLB	% of named fatty acid	Ester composition %		Form
				Mono ester	Di, tri, poly ester	
Sucrose laurate	C-1201	1	95	1	99	Liquid
	C-1216	16	95	80	20	Pellet
Sucrose myrisate	C-1416	16	95	80	20	Powder
Sucrose palmitate	C-1616	16	80	80	20	Powder
Sucrose stearate	C-1805	5	70	30	70	Powder
	C-1807	7	70	40	60	Powder
	C-1811	11	70	55	45	Powder
	C-1815	15	70	70	30	Powder
	C-1816	16	70	75	25	Powder

6.3.6. Polysorbate 80

It is commercially known as Tween™ 80 which is a non-ionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. It is a viscous, water-soluble clear yellow oily liquid with slight fatty odor (Polysorbate 80 product information, 2009). The melting point is -20.56°C. Polysorbate 80 is primarily used in food, cosmetics and beauty products as a surfactant and emulsifier because of its ability to help other ingredients dissolve in a solvent in which they normally would not be able to dissolve, specifically in the case of oil in water. It can also help to reduce surface tension of substances that need to be emulsified. It is seen in a variety of formulas, including skin fresheners, skin care products, skin cleansing products, makeup bases and foundations, shampoos, and fragrance powders, as well as food products. It is also a popular ingredient in ice cream, because of its ability to prevent milk proteins from completely coating the fat droplets. This allows them to join together in chains and nets, to hold air in the mixture, and provide a firmer texture, holding its shape as the ice cream melts. This process is similar to the above mentioned ability of polysorbate to reduce surface tension in cosmetic and skin care formulas. Polysorbate 80 is Cosmetic Ingredient Review (CIR) and FDA approved. It is low toxicity and can be used by intravenous administration. In pharmaceutical application, Polysorbate 80-coated polybutyl cyanoacrylate nanoparticles were shown to enable the transport of a number of drugs including the anti-tumour antibiotic doxorubicin across the blood brain barrier to the brain after intravenous administration and to considerably reduce the growth of brain tumours in rats (Gelperina and others, 2002). Another study found out that nanoparticles overcoated by Polysorbate 80 could significantly improve the drug level in both brain tissues and

cerebrospinal fluids compared with uncoated ones and simple solution. Polysorbate 80-coated polybutylcyanoacrylate nanoparticles could be used to overcome blood brain barrier especially those whose diameter was below 100 nm (Gao and Jiang, 2006).

6.3.7. Dermofeel^{TM} SL

The INCI name is sodium stearoyl lactylate. Dermofeel^{TM} SL is a vegetable food grade anionic co-emulsifier for o/w emulsions (Dermofeel^{TM} SL product information, 2010). Dermofeel^{TM} SL is multi-purpose cosmetic additive and can be applied as emulsifier (Gomez and others, 2004; Manohar and Rao, 1999; Swanson and others, 1999), whipping agent (Kelly and others, 1999) and conditioning agent (Armero and Collar, 1998) in a wide variety of modern food technologies. The high emulsifying efficiency of sodium stearoyl lactylate is based on its amphiphilic nature consisting of a hydrophilic charged head and long hydrophobic hydrocarbon tail. Due to its amphiphilic character it can be used as co-emulsifier, co-surfactant, or hair care products.